A tribute to Akira Endo, discoverer of a “Penicillin” for cholesterol
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It is an honor to play tribute to Akira Endo, whose discovery of statins changed the world. We first became aware of Endo in July 1976 when he published a review article in Japanese, describing our recent discovery of the LDL receptor and its role in the regulation of cholesterol metabolism in human fibroblasts [1]. Although we could not understand the Japanese words, we were amazed to see that Endo had reproduced five of our figures, introducing our work to a Japanese audience. The journal *Seikagaku* (Journal of the Japanese Biochemical Society) is not on our normal reading list, and the Internet did not exist. How did we learn about Endo’s article?

At that time we subscribed to a computer-based service from the Institute for Scientific Information (ISI) that tracked all of the scientific articles that cited our work. Each week, we received a computer printout of these articles, and in July 1976 Endo’s review article appeared on our list. In as much as our library did not subscribe to *Seikagaku*, we ordered Endo’s article directly from the ISI. In order to track Endo’s future work more closely, we added his name to the list of authors that the ISI screened for us. In early 1977, the ISI informed us of two new articles by Endo, one in *The Journal of Antibiotics* [2] and one in *FBBS Letters* [3]. We obtained copies of these articles, and we were amazed to find that Endo had discovered a molecule, which he called ML-236B, that was a potent competitive inhibitor of HMG CoA reductase [2,3]. Had we not subscribed to the ISI computer service, it may have been years before we would have heard of Endo and his historical discovery.

In the years preceding 1976, the literature was full of papers describing molecules that were claimed to be inhibitors of HMG CoA reductase. They ranged from oleic acid to cyclic AMP. All of the previous inhibitor studies were suspect, either because of the high concentrations of compounds required, or because the experiments were poorly controlled. Endo’s work stood out for three reasons: (1) ML-236B was very potent; it worked at 10 nM; (2) he performed kinetic experiments showing that ML-236B acted competitively with respect to the substrate HMG CoA; and (3) ML-236B has a five-member lactone ring that resembles mevalonate, the product of the HMG CoA reductase enzyme, thus supporting the observation that ML-236B is a competitive inhibitor.

We immediately wrote to Endo and asked him for a sample of ML-236B that we could use in tissue culture studies of cultured human fibroblasts [4]. We had developed a system in which we could measure the activity of HMG CoA reductase in extracts from these cells, and we had shown that the enzyme activity declines dramatically when cholesterol enters the cell through LDL receptors [5]. We wanted to know what would happen to cholesterol metabolism if we inhibited the enzyme competitively, and not through the delivery of cholesterol. Endo promptly and generously sent us 1 g of ML-236B and wrote that he planned to attend the 6th International Symposium on Drugs Affecting Lipid Metabolism (DALM), which was to be held in Philadelphia [6]. We invited him to visit our laboratory in Dallas on his way home from Philadelphia, and he graciously accepted. When Dr. Endo arrived in Dallas on September 2, 1977, he was depressed: his presentation at DALM had aroused very little interest. The field was mesmerized by fibrates and bile acid sequestrants. Almost no one came to hear his talk. On the other hand, we were very excited because we realized that ML-236B would be a powerful experimental tool, and we even proposed to Endo that we do a clinical trial in patients with hypercholesterolemia [4]. Endo declined our offer, and the honor of the first clinical trial went to Akira Yamamoto [7].

In his original paper [2], Endo mentioned that a molecule identical to ML-236B had been isolated previously (and given the name of compactin) by a group at Beecham Pharmaceutical Research Laboratories in England [8]. They had purified compactin from another strain of penicillin mold because of its anti-gonococcal activity in vitro. But they lost interest when the drug was not potent enough to be used as an anti-gonococcal therapeutic agent. The Beecham investigators happily sent us 500 mg of compactin.
bind I-LDL with high affinity. Endo had shown that liver, and we had shown that bovine liver membranes had shown that the majority of LDL clearance occurs in the and adrenal cells. By 1979, Daniel Steinberg and co-workers normal gene.

Would this increase lead to a lowering of LDL in plasma? by compactin also increase the number of LDL receptors? We already knew that LDL receptors were subject to the same type of end-product feedback repression as the product of HMG CoA reductase in response to compactin had an enormous effect on our thinking. It meant that production of the enzyme was under constant feedback repression by the end-product, cholesterol. Compactin relieved that repression. We already knew that LDL receptors were subject to the same type of end-product feedback repression by cholesterol. Would the deprivation of cholesterol by compactin also increase the number of LDL receptors? Would this increase lead to a lowering of LDL in plasma? Our focus on LDL receptors was reinforced strongly by Watanos 1980 paper in which he showed that FH heterozygotes responded well to compactin, but two FH homozygotes showed marked resistance to the drug. This was to be expected if compactin acts by stimulating the LDL receptor gene. FH heterozygotes have one normal gene that can be stimulated, whereas receptor-negative homozygotes have no normal gene. Our earlier studies were performed in cultured fibroblasts and adrenal cells. By 1979, Daniel Steinberg and co-workers had shown that the majority of LDL clearance occurs in the liver, and we had shown that bovine liver membranes bind I-LDL with high affinity. Endo had shown that compactin treatment causes a large increase in HMG CoA reductase in rat livers. Toru Kita, our first Japanese post-doctoral fellow, showed that this increase could be reversed by administration of mevalonate, the product of HMG CoA reductase. All of these results in liver were identical to those in fibroblasts, and we postulated that statins would increase LDL receptors in liver, as they did in cultured cells. We could not test this hypothesis in rats and mice because they had almost no LDL in plasma. Endo had informed us that compactin lowered LDL in dogs. At that time, we were consultants to Merck Sharp & Dohme Research Laboratories where Roy Vagelos had become head of research in 1973. Vagelos and his assistant Al Alberts, had a longstanding interest in lipid synthesis from their days at Washington University, and they carried this interest to Merck. In 1977, we sent Vagelos a preprint of our joint paper with Endo, and we included a letter that proposed a formal collaboration between our laboratory and Merck in the clinical development of statins. Taking advantage of the observation that compactin was isolated from a penicillin mold, we proposed that compactin would become a "penicillin" for cholesterol. Merck never agreed to a formal collaboration, but they did keep us working as consultants, and we made frequent visits to their laboratories throughout the development of the statins.

By 1980, Merck had discovered lovastatin, which was then called mevinolin. Endo had discovered the same compound, which he called monacolin-K. Like Endo, Alberts had shown that lovastatin lowers LDL levels in dogs. After some pleading, Alberts agreed to supply us with a small amount of lovastatin for our studies. In fact, our supply of lovastatin was so limited that we had to carry out the experiments in very young Beagle dogs. The studies were conducted in 1980 with Pietro Kovanen, a postdoctoral fellow in our laboratory, and David Bilheimer, a faculty colleague. The results were dramatic. When we gave lovastatin to dogs, LDL receptor activity in liver was up-regulated, as revealed by assays of the binding of I-LDL to liver membranes, and clearance of I-LDL from plasma was accelerated. The relative decline in plasma LDL was much greater than the decline in HDL, which does not bind to LDL receptors. The effectiveness of lovastatin in raising LDL receptors and lowering plasma LDL was enhanced by the simultaneous administration of cholestyramine, a bile acid-binding resin that also depletes the liver of cholesterol and leads to up-regulation of LDL receptors. These results were published in the Proceedings of the National Academy of Sciences in 1981.

Our results in dogs convinced us that lovastatin had the potential to lower LDL in humans whose LDL receptors were suppressed by the combination of dietary and endogenously synthesized cholesterol. If endogenous synthesis could be partially blocked, one might observe an up-regulation of LDL receptors and lowering of LDL.

As others have noted, Sankyo halted the clinical trials of compactin in 1980 because of a fear that the drug caused tumors in dogs. When this observation became known, Merck abandoned its pursuit of lovastatin. Fortuitously, at this point one of us (Goldstein) made a trip to Japan. Goldstein visited
Endo in his laboratory at Tokyo Nokonos Medical Institute (Endo had left Sankyo in December 1978), and he found Endo again to be in a state of depression. Endo told Goldstein that he did not believe that compactin caused tumors. Rather, he said that the dogs had been given extremely high doses of compactin so that they essentially had no LDL in their plasma for two years. At autopsy the pathologists saw abnormally stained cells in the intestine which they interpreted to be malignant lymphoma. However, Endo believed that the microscopic changes reflected a toxic reaction to the large amounts of undigested drug in the intestinal lumen.

Endo’s comments about abnormal staining struck a chord with us. We had observed that when cultured cells were treated with high levels of compactin, the amount of HMG CoA reductase increased, and this caused a massive increase in the endoplasmic reticulum (ER), the organelle that houses HMG CoA reductase. When the amount of ER increased markedly, the cells developed crystalline membrane tubules that stained abnormally with various dyes. This abnormal staining might explain the pathologist’s mistaken interpretation of malignancy.

Armed with this information, we urged Merck to continue the development of lovastatin. Al Alberts had never lost his faith in the compound, but some of the top executives were fearful of lovastatin, and Vagelos was uncertain. Some of the Merck scientists thought that lovastatin was tumorigenic because of some intrinsic property of the molecule, and not because it inhibits HMG CoA reductase. They therefore were urgently seeking another compound that would inhibit HMG CoA reductase, but not have the tumorigenic property.

We believed that the abnormally stained cells were a result of HMG CoA reductase inhibition and that any inhibitor of the reductase would produce the same result when given at extremely high doses. We therefore challenged Merck to do the following experiment [22]: Administer lovastatin to dogs with or without mevalonate, the product of the enzyme. If the toxicity is due to excessive inhibition of HMG CoA reductase, mevalonate should relieve the toxicity. On the other hand, if toxicity were caused by an idiosyncrasy of the lovastatin molecule, then mevalonate should have no effect. If the toxicity were caused by excessive inhibition of HMG CoA reductase, this should not preclude use of the drug since one would never administer it to humans in doses high enough to reduce LDL to zero.

Our confidence in statins was strongly reinforced when we saw Mabuchi’s 1981 paper in The New Zealand Journal of Medicine on the treatment of FH heterozygotes with compactin [23]. Heterozygotes have one copy of a normal gene for LDL receptors, but this gene is partially suppressed by cholesterol. Compactin relieves this suppression, up-regulating LDL receptors and lowering plasma LDL. We communicated our excitement in the editorial that we were invited to write to accompany Mabuchi’s article, which was entitled “Lowering Plasma Cholesterol by Raising LDL Receptors” [24]. Although we were enthusiastic, we cautioned that many hurdles had to be overcome before compactin would be a drug. Among these was the ever-present concern about long-term toxicity, a concern that was not laid to rest until Merck conducted the landmark EXCEL study after the approval of lovastatin [25].

At this point, the notion that statins act in humans by raising LDL receptors was a hypothesis. We needed direct evidence. The way to get this direct evidence was to do a turnover study with 125I-LDL in humans. David Bilheimer and his colleagues at NIH had developed these methods, and Bilheimer was now a colleague of ours in Dallas. By this time, a leading practitioner of this art was Scott Grundy, who was then at the University of California, San Diego. Grundy was a Texan, and we had the temerity to think that we could recruit him back to his native state to head a new nutrition center that had just been established in Dallas. In his tribute to Endo in the Proceedings of the 13th International Symposium on Atherosclerosis (Kyoto, 2003), Grundy describes the lunch that we had with him and Bilheimer where we planned the studies of 125I-LDL turnover in lovastatin-treated patients. Grundy and Bilheimer agreed to collaborate on those studies, and the results were published in the Proceedings of the National Academy of Sciences in 1983 [26] and presented at the plenary session of the American Association of Physicians on April 29, 1983. The results demonstrated that statins enhanced plasma clearance of native LDL, but not LDL that had been modified so that it no longer bound to LDL receptors. Clearly, statins were working by up-regulating receptors. Bilheimer and Grundy went on to show that receptor-negative FH homozygotes, who had no LDL receptors to up-regulate, failed to respond to high doses of lovastatin [27].

During the early 1980’s, Merck continued to vacillate about the clinical development of lovastatin. After a great deal of argument back and forth, Merck finally decided to complete its development. The final decision was made when Edward Scolnick became head of research. We had admired Scolnick since our residency days together at Massachusetts General Hospital. On March 18, 1984, Scolnick made a special trip to Dallas [28], so that we could explain to him our theory of the mechanism by which lovastatin could lower LDL specifically and without toxicity. We believe that this information, together with unfailing pressure from Alberts, caused Merck to reinstitute its lovastatin development program. As you all know, lovastatin was commercialized by Merck in 1987 as the first statin to be approved for human therapy.

None of this would have happened if Endo had not conducted his relentless search for a fungal product that would inhibit cholesterol synthesis, and if he had not shown convincingly that ML-236B inhibited HMG CoA reductase. Without Endo, the statins might never have been discovered, and without Alberts, Vagelos, and Scolnick at Merck, statins might never have become approved drugs. Shortly after Endo’s work, pharmaceutical companies turned away from screening natural products. Instead, they now use synthetic chemical libraries. No random chemical library would ever yield an HMG-CoA reductase inhibitor as potent as the natural statins.
The millions of people whose lives will be extended through statin therapy owe it all to Akira Endo and his search through fungal extracts at the Sankyo Co.

References

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